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Structural characterization of recombinant human fibroblast growth factor receptor 2b kinase domain upon interaction with omega fatty acids.

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Abstract

The mutated recombinant kinase domain of human fibroblast growth factor receptor 2b (hFGFR2b) is overexpressed and purified, and its structural changes upon the interaction with three unsaturated fatty acids (UFAs), oleic, linoleic and α-linolenic are studied. This interaction is investigated to find out about the folding and unfolding effect of unsaturated fatty acids on the kinase domain structure of hFGFR2b. Recombinant pLEICS-01 vectors, containing the mutated coding region of hFGFR2b, are expressed in the standard Escherichia coli BL21 (DE3) host cells and purified by Ni^{2+}-NTA affinity chromatography. While polyacrylamide gel electrophoresis characterizes the functionality of recombinant protein, its structural changes are studied in the presence and absence of various concentrations of oleic, α-linolenic and linoleic acids using circular dichroism (CD) and fluorescence spectroscopy. Far ultraviolet CD results show that unsaturated fatty acids do not change the secondary structure of the recombinant kinase domain of hFGFR2b. However, chemical denaturation analysis confirms that all three UFAs destabilize the tertiary structure of recombinant protein. A decrease in the fluorescence intensity without any significant red or blue shift (336±1nm) reflects a variation in the tertiary structure of protein. The direct interaction of the studied UFAs with hFGFR2b reduces the conformational stability of their kinase domains. The structural changes in hFGFR2b in the presence of UFAs may be necessary for hFGFR2b to adjust the signal transduction and regulate the key cellular processes.

KEYWORDS: Chemical denaturation; Kinase domain; Tertiary structure; Unsaturated fatty acids; hFGFR2b

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